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(54) Title: **BIOMATERIALS BASED ON HYALURONIC ACID FOR THE ANTI-ANGIOGENIC THERAPY IN THE TREATMENT OF TUMOURS**

(57) Abstract: The use in the medical-surgical field of biomaterials based on hyaluronic acid derivatives, optionally in association with natural, synthetic or semisynthetic biopolymers, for suppressing the angiogenic process associated with tumour proliferation (in primary and secondary tumours) is disclosed.

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**BIOMATERIALS BASED ON HYALURONIC ACID FOR THE ANTI-  
ANGIOGENIC THERAPY IN THE TREATMENT OF TUMOURS**

**SUBJECT OF THE INVENTION**

The present invention relates to the use in the medical-surgical field of biomaterials based on hyaluronic acid derivatives, optionally in association with natural, synthetic or semisynthetic biopolymers, for suppressing the angiogenic process associated with tumour proliferation (in primary and secondary tumours).

**BACKGROUND OF THE INVENTION**

The induction and development of angiogenesis is a pre-requisite for the development of a primary tumour, and for any subsequent metastases.

Angiogenesis is a dynamic process closely linked with the proliferation of cancer cells, because it is the latter that are chiefly responsible for the production and release of angiogenic factors, such as cytokines and other trophic factors. An increase in the vascularisation of a primary tumour can cause an increase in the number of cancer cells that enter into the circulation and give rise to new metastases.

Recent studies have demonstrated that an increase in the density of microvessels in an area affected by neoplasia indicates new tumour growth.

It is therefore clinically important to suppress angiogenesis to inhibit its development, if possible. Indeed, by associating anti-angiogenic therapy with "classic" anticancer therapy with drugs and/or radiation, with or without surgical removal of the tumour, it is possible to halt the proliferation of cancer cells, thus preventing the invasion of further tissues by said cells, and the consequent development of new metastases (Skobe H. et al., Nature Medicine, 1222-1227 (1997)).

In histological assessment of the onset of the angiogenic process

associated with a cancerous growth, it is important to look for markers of the tumour's vascular system, for example with antibodies that differentiate the endothelial cells from the cancerous ones. For example, the anti-CD3 antibody is specific for marking the endothelial cells and therefore enables their  
5 identification in the angiogenic process associated with the development of new metastases. Its use has proved essential in assessing the level of microvessel development associated with neoplasia. Indeed, thanks to antibody marking, it is possible to visualise and count the number of interconnections of the vessels within the cancerous tissue to understand and  
10 quantify the angiogenic process, relating it to any new developments in the neoplasia (thereby deciding if/how much/how to associate a therapy that modulates or inhibits angiogenesis with an established/classic anticancer therapy.

One such therapy consists in administering drugs that act by blocking  
15 the receptors of the trophic factors (PGDF, bFGF, VEGF) that are also angiogenic factors.

Preclinical results 'in vivo' have shown that said drugs prove important in inhibiting tumour growth but they do not determine regression of the tumour itself: on the strength of these major experimental data, the drugs have  
20 been introduced in numerous clinical trials.

However, an anti-angiogenic clinical therapy that provides for a generally oral pharmacological administration in chronic form may have many toxic side effects, because angiogenesis is not only associated with pathological disorders but also physiological processes such as tissue  
25 reproduction and repair ("*Cancer: Principle Practice of Oncology*" V. De Vita, S. Hellmann and S. Rosenberg, 6<sup>th</sup> Edition).

It is therefore of strategic importance to associate classic anticancer therapy with an anti-angiogenic therapy "in situ", and this is the subject of the

present invention.

Hyaluronic acid is one of the chief components of the extracellular matrix of the connective tissue, and there are numerous scientific publications concerning its role in various processes, both physiological and pathological, such as the formation of granulation tissue, chemotaxis in the inflammatory process, cell differentiation for various cell types. Other studies concern its role within the family of "substrate adhesion molecules".

Hyaluronic acid has been used for the above indications:

- as a differentiating agent in therapy for acute myeloid leukaemia (Charrad R. S. et al., Nature Medicine 5, 669-676 (1999));
- as a vehicle for drugs such as steroids or NSAIDs, antibiotics and anti-neoplastic agents, because of the abundant expression of its receptor (CD44) in cancer cells; (Freemantle, C. et al., Int. J. Tiss. Reac. XVIII (4) 157-166 (1995); Coradini, D. et al., Int. J. Cancer 5, 411-416 (1999));
- in preclinical studies on the inhibition of lung metastasis, because of its capacity for inhibiting the adhesion of cancer cells to the vascular endothelium (Karasaza K. et al., Clinical & Experimental Metastasis 15, 83-93 (1997));
- as a means of controlling adhesion to the substrate with subsequent proliferation of cells (possibly also cancer cells) permanently "in situ" after surgical removal of tissues (including tumours) (U.S. 5,627,162).

Experimental observations "in vivo" have, however, revealed that hyaluronic acid may have a chemotaxic activity on cancer cells within the granulation tissue that forms after removal of cutaneous metastasis of melanoma (Salmon-Ehr, V. et al., Ann. Dermatol. Venereol, 123, 194-195 (1996)). Moreover, numerous pre-clinical studies have demonstrated that

hyaluronic acid enhances cancer cell migration, thereby favouring metastasis, as it is known that the degradation products of hyaluronic acid, that is, oligosaccharides constituted by 10 and 20 oligomers, are strong inducers of the angiogenic process (Hayen et al., J. Cell. Sci. 112, 2241-2251 (1999); Slevin, M. et al., Lab. Invest. 78(8), 987-1003 (1998)).

Moreover, biomaterials based on hyaluronic acid and/or the derivatives thereof have never been used as an anti-angiogenic therapy, neither have any other biodegradable and/or non-biodegradable biopolymers ever been used in anticancer therapies.

Absolutely innovative, therefore, is the use of biomaterials based on hyaluronic acid derivatives such as Hyaff® (EP 0 216 453 B1) or ACPs (EP 0 341 745 B1) in the form of non-woven felts for instance (EP0 618 817 B1) or as three-dimensional structures (WO 99/61080), possibly in association with various biomaterials (e.g. natural ones such as collagen, cellulose, polysaccharides, chitin, chitosan, pectin, agar, gellan and alginic acid, synthetic ones such as polylactic acid (PLA), polyglycolic acid (PGA), polyurethanes and polysulphonic resins, or semisynthetic ones such as collagen cross-linked with aldehyde, diamine and gellan) as a therapy to suppress and/or inhibit the angiogenic process that enhances and determines tumour metastasis.

## DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to biomaterials based on hyaluronic acid derivatives made into non-woven felts (as the preferred form of biomaterial), optionally in association with natural, synthetic or semisynthetic biopolymers, for use in the medical-surgical field as a new anti-angiogenic therapy ("in situ"), optionally associated with classic pharmacological anticancer therapies and/or radiotherapy, to modulate indirectly the proliferation of tumours, thus blocking the formation of local relapses and, therefore, any new metastases.

In order to study, characterise and then assess “in vivo” the effect of the biomaterial of the present invention in the angiogenic process that supports the development of skin carcinomas (considered to be a clarifying example), the Applicant has developed a new model of tumour/ stromal cell support interaction, described as follows:

- 1) two cell lines of human keratinocytes transfected with the ras-oncogene: HACaT II-4, malignant variant and A5, benign variant;
- 2) said cells are transferred onto a collagen gel mounted into teflon rings covered by a silicone chamber, known as the Fusenig silicone chamber (FSC);
- 3) said FSC is then placed over the muscle fascia of the backs of nude mice, in the presence or absence of an immediately underlying layer of biomaterial based on Hyaff® 11 (total benzyl ester of hyaluronic acid) made in the form of a non-woven felt;
- 4) four to six weeks later, two different types of granulation tissue will have formed underneath the cancerous epithelium;
- 5) the development of the epithelial tumour and of the underlying granulation tissue is assessed, over time, both by classic histological analyses (haematoxylin/eosin) and by immunohistochemical techniques using the anti-CD31 antibody, to visualise the presence of vascular epithelium and therefore determine the development of the angiogenic process;
- 6) the levels of cellular proliferation are examined using immunohistochemical techniques associated with the introduction of BrdU into the DNA of proliferating cells, both within the granulation tissue underneath the epithelium and in the cancerous epithelium itself. Marking with the anti-integrin  $\alpha 6$  antibody was also assessed to study the level of cellular proliferation within the

cancerous epithelium.

The results of the experiment were as follows:

HACaT A5 line:

After 4-6 weeks, the cancerous epithelium in the control FSC (i.e.  
5 without any biomaterial placed under the epithelium), was well developed and  
multilayered, while the layer of granulation tissue underneath had completely  
replaced the layer of collagen that separated the epithelium from the  
underlying tissue (Fig. 1).

Conversely, four weeks later, the cancerous epithelium in the FSC  
10 placed over the Hyaff®-based biomaterial in the form of a non-woven felt is  
less developed than the relative control, and the layer of collagen that  
separates it from the nascent granulation tissue underneath is still thick and  
not infiltrated by cells and/or vessels (Fig. 1).

After six weeks, the quantity of collagen is still abundant, with just an  
15 initial layer of granulation tissue that begins to form over the biomaterial (Fig.  
1).

HACaT II 4 line:

After four to six weeks, in the control FSC, the cancer cells have  
constituted a thick epithelium that penetrates into the thickness of the new  
20 granulation tissue underneath, that has already completely replaced the layer  
of collagen that separated it from the epithelium (Fig. 2).

Four weeks later, in the FSC placed over the Hyaff®-based biomaterial,  
the cancerous epithelium is thin but easily distinguishable from the  
granulation tissue forming over the biomaterial, separated from this tissue by  
25 the collagen gel that is still present and not yet absorbed (Fig. 2).

Six weeks later, the tumour mass and the granulation tissue have  
established close contact, but there has been no actual infiltration of tumour  
cells into the granulation tissue, unlike the control, where the tumour cells

have completely invaded the new, underlying granulation tissue (Fig. 2).

Using immunohistochemical techniques linked with the specific marking of particular nucleotides such as BrdU, at the 1<sup>st</sup> and 2<sup>nd</sup> weeks, good cell proliferation is evident within the nascent granulation tissue in the control  
5 and in the Hyaff®-based biomaterial, while at 4, and especially at 6, weeks after transplant, the cell growth rate drops drastically in the granulation tissue underneath the cancerous epithelium, which conversely maintains in both samples a good level of cellular proliferation (Fig. 3).

The growth of cancerous epithelium can also be visualised with a  
10 specific antibody against the protein integrin  $\alpha 6$ . Said molecule is, indeed, a component of the hemidesmosomes and its expression is normally only associated with the proliferative area of the epithelial layers.

Fig. 3a shows that antibody marking for the integrin protein  $\alpha 6$  is notably present throughout the cancerous epithelium both in the control FCS  
15 and in the FCS with the Hyaff®-based biomaterial, even though expression of the test protein appears less extensive throughout the thickness of the cancerous epithelium in the latter sample.

Specific marking for the vascular epithelium with the anti-CD31 antibody reveals, furthermore, that in the controls, after four weeks, the  
20 angiogenic process is well established as the vessels in the underlying granulation tissue already reach the cancerous epithelium and after 6 weeks they invade it, thus favouring metastasis (Fig. 4).

In the case of the FSC with the Hyaff®-based biomaterial, after four weeks there is still no close contact between granulation tissue and cancerous  
25 epithelium. This will occur only after six weeks, even though there is no invasion of the epithelium by the underlying microvessels, that remain relegated to the granulation tissue (Fig. 4).

The angiogenic process seems to be at a standstill, no longer enhancing



tumour development. Vascularisation is limited to the area covered by the Hyaff®-based biomaterial, so the tumour cells do not invade the granulation tissue that has formed within the biomaterial.

In conclusion, the Hyaff®-based biomaterial proved able to modulate/inhibit the angiogenic process related to vascularisation of the cancerous epithelium. It therefore proves to be particularly advantageous to use the biomaterials based on hyaluronic acid derivatives in the oncological field, where it is important to modulate the angiogenic process and therefore, indirectly, the proliferation of cancer cells in primary and secondary tumours.

According to the invention, the biomaterials that can be useful in the oncological field as a new anti-angiogenic therapy "in situ" may be, for example, in the form of non-woven felts, sponges, films, membranes, microspheres or in other three-dimensional forms in cases where it is necessary to fill the cavities that are liable to form after surgical removal of a tumour.

The anti-angiogenic action of the biomaterial can, moreover, be supported by supplementing the biomaterial with NSAIDs, steroids, hormones, antibiotics and especially anti-cancer drugs such as fluorouracil, methotrexate, cis-platinum, carboplatin, oxaliplatin, etoposide, cyclophosphamide, vincristine, doxorubicin.

The invention being thus described, it is clear that these methods can be modified in various ways. Said modifications are not to be considered as divergences from the spirit and purposes of the invention and any modification that would appear evident to an expert in the field comes within the scope of the following claims.

**CLAIMS**

1. Biomaterials constituted by at least one hyaluronic acid derivative, optionally in association with other natural, synthetic and/or semisynthetic  
5 biopolymers and with pharmacologically active substances, as an anti-angiogenic therapy to treat primary and secondary tumours.
2. Biomaterials according to claim 1, wherein the hyaluronic acid derivative is a benzyl ester.
3. Biomaterials according to claim 1, wherein the hyaluronic acid  
10 derivative is cross-linked.
4. Biomaterials according to claim 1, wherein the natural biopolymer is selected from the group consisting of collagen, cellulose, polysaccharides, chitin, chitosan, pectins, agar, gellan and alginic acid.
5. Biomaterials according to claim 1, wherein the synthetic biopolymer is  
15 selected from the group consisting of polylactic acid (PLA), polyglycolic acid (PGA), polyurethanes and polysulphonic resins.
6. Biomaterials according to claim 1, wherein the semisynthetic biopolymer is selected from the group consisting of collagen cross-linked with aldehydes, diamine and gellan.
- 20 7. Biomaterials according to claim 1, wherein the biopolymer may optionally be in association with pharmacologically active substances such as fluorouracil, methotrexate, cis-platinum, carboplatin, oxaliplatin, ethopoxide, cyclophosphamide, vincristine, doxorubicin.
8. The use of biomaterials constituted by at least one hyaluronic acid  
25 derivative, optionally in association with other natural, synthetic and/or semisynthetic biopolymers and with pharmacologically active substances, as an anti-angiogenic therapy for the treatment of primary and secondary tumours.

9. The use of biomaterials according to claim 8, wherein the hyaluronic acid derivative is a benzyl ester.

10. The use of biomaterials according to claim 8 wherein the hyaluronic acid derivative is cross-linked.

5 11. The use of biomaterials according to claim 8 wherein the natural biopolymer is selected from the group consisting of collagen, cellulose, polysaccharides, chitin, chitosan, pectins, agar, gellan and alginic acid.

12. The use of biomaterials according to claim 8 wherein the synthetic biopolymer is selected from the group consisting of polylactic acid (PLA),  
10 polyglycolic acid (PGA), polyurethanes and polysulphonic resins.

13. The use of biomaterials according to claim 8 wherein the semisynthetic biopolymer is collagen cross-linked with aldehydes, diamine and gellan.

14. The use of biomaterials according to claim 8 wherein the biopolymer may optionally be in association with pharmacologically active substances  
15 such as fluorouracil, methotrexate, cis-platinum, carboplatin, oxaliplatin, ethopoxide, cyclophosphamide, vincristine and doxorubicin.

15. The use of a biomaterial according to claim 8 wherein the hyaluronic acid derivative is made into a non-woven felt, sponge, microsphere, film, membrane and/or other three-dimensional structures.

20 16. The use of biomaterials constituted by at least one hyaluronic acid derivative, optionally in association with other natural, synthetic and/or semisynthetic biopolymers and with pharmacologically active substances, for the treatment and care of primary and secondary tumours when the tumour has been surgically removed and the cavity that is thus formed requires filling,  
25 making it advantageous to prevent and/or inhibit the angiogenic process using the biomaterial itself.

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A5



A5 Hyaff 11



4



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**Fig. 1**

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II-4



II-4 Hyaff 11



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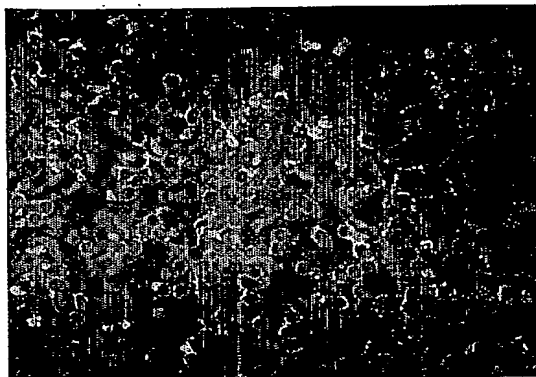
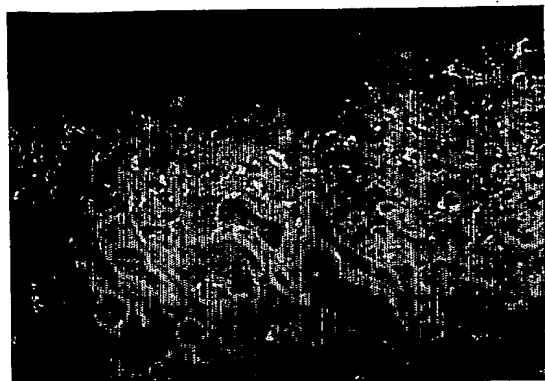


**Fig. 2**

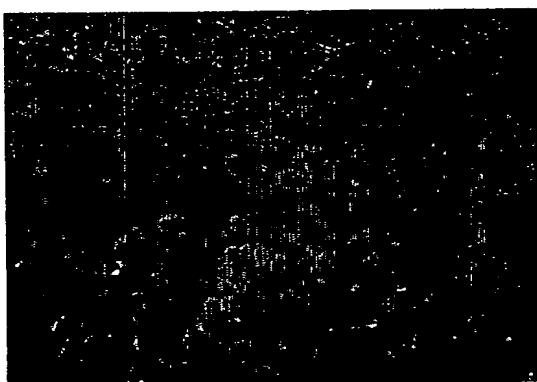
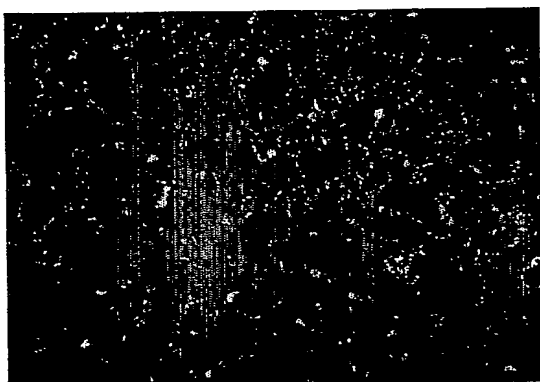
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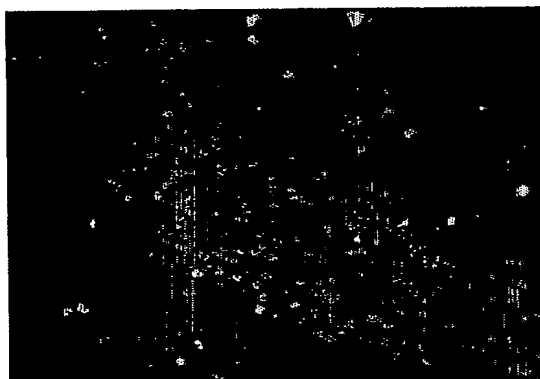
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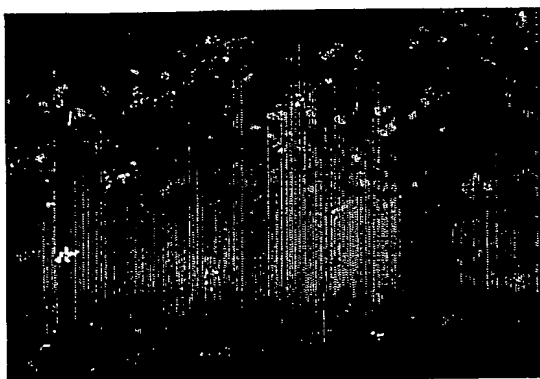
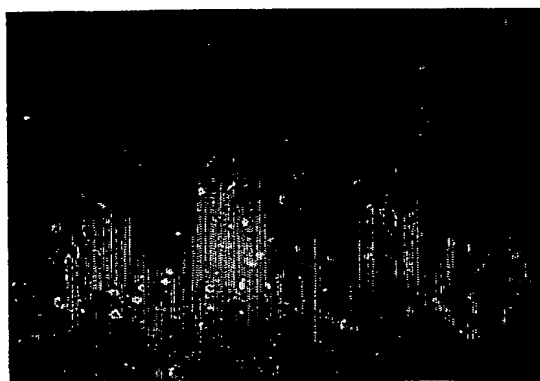
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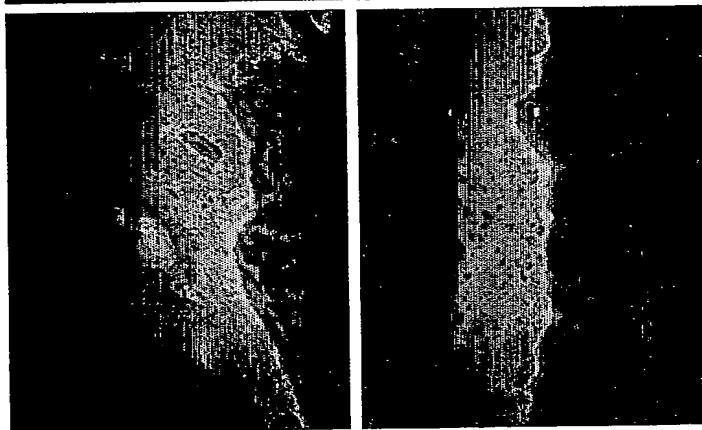
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**Fig. 3**

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II-4

II-4 Hyaff 11

Fig. 3a

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II-4 Hyaff 11

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**Fig. 4**



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Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

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## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P, X	WO 02 18448 A (FRANCESCANGELI ANDREA ; BELLINI DAVIDE (IT); CRESCENZI VITTORIO (IT) 7 March 2002 (2002-03-07) page 3, line 9-12 claims 5, 11, 15, 24 ---	1-16
P, X	WO 02 41877 A (CLEAR SOLUTIONS BIOTECH INC ; DEHAZYA PHILIP (US); LU CHENG (US)) 30 May 2002 (2002-05-30) page 4, line 19-22 page 18, line 8 page 16, line 21 ---	1-16
P, X	WO 02 18450 A (FRANCESCANGELI ANDREA ; RENIER DAVIDE (IT); CRESCENZI VITTORIO (IT)) 7 March 2002 (2002-03-07) page 2, line 10-12 page 10, line 27 --- -/--	1-16



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## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 0 341 745 A (FIDIA SPA) 15 November 1989 (1989-11-15) page 1, line 32 page 5, line 41-49 page 6, line 19-28 page 9, line 55 page 10, line 11 page 10, line 14 ---	1-7
X	WO 99 61080 A (CALLEGARO LANFRANCO ; DONA MASSIMO (IT); FIDIA ADVANCED BIOPOLYMERS) 2 December 1999 (1999-12-02) page 1, line 6-13 page 4, line 23-28 ---	1-7
X	US 5 520 916 A (CALLEGARO LANFRANCO ET AL) 28 May 1996 (1996-05-28) column 1, line 8-13 claim ALL ---	1-7
X	WO 00 01733 A (BELLINI DAVIDE ; FIDIA ADVANCED BIOPOLYMERS SRL (IT); TOPAI ALESSAN) 13 January 2000 (2000-01-13) page 1, line 11-14 claims 1,5,16,21,23 ---	1-16
X	WO 00 54762 A (UNIV BOSTON ; MOULTON STEVEN (US)) 21 September 2000 (2000-09-21) claims 1-31 ---	1-16
X	WO 00 57896 A (MUMMERT MARK E ; TAKASHIMA AKIRA (US); UNIV TEXAS (US); MOHAMADZADE) 5 October 2000 (2000-10-05) page 3, line 21-32 ---	1-16
X	US 4 851 521 A (DELLA VALLE FRANCESCO ET AL) 25 July 1989 (1989-07-25) column 1, line 6-12 claims 1-8,25 ---	1-16
X	EP 0 466 300 A (BIOMATRIX INC) 15 January 1992 (1992-01-15) page 3, line 35-47 page 7, line 28-31 ---	1-16
P,X	TONELLO C ET AL: "In vitro reconstruction of human dermal equivalent enriched with endothelial cells" BIOMATERIALS, ELSEVIER SCIENCE PUBLISHERS BV., BARKING, GB, vol. 24, no. 7, March 2003 (2003-03), pages 1205-1211, XP004401497 ISSN: 0142-9612 abstract --- -/--	1-7

## INTERNATIONAL SEARCH REPORT

International Application No

PCT/EP 03/00078

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	GLASS J ET AL: "A three-dimensional cell attachment matrix created by cross-linking RGD peptide modified hyaluronic acid" JOURNAL OF CELLULAR BIOCHEMISTRY - SUPPLEMENT, WILEY-LISS, US, no. SUPPL 19A, 5 January 1995 (1995-01-05), page 178 XP002112805 ISSN: 0730-2312 abstract ---	1-7
X	LUO Y ET AL: "Cross-linked hyaluronic acid hydrogel films: new biomaterials for drug delivery" JOURNAL OF CONTROLLED RELEASE, ELSEVIER SCIENCE PUBLISHERS B.V. AMSTERDAM, NL, vol. 69, no. 1, 3 October 2000 (2000-10-03), pages 169-184, XP004217543 ISSN: 0168-3659 abstract ---	1-7
X	CORADINI D ET AL: "Hyaluronic acid as drug delivery for sodium butyrate: improvement of the anti-proliferative activity on a breast-cancer cell line." INTERNATIONAL JOURNAL OF CANCER. JOURNAL INTERNATIONAL DU CANCER. UNITED STATES 5 MAY 1999, vol. 81, no. 3, 5 May 1999 (1999-05-05), pages 411-416, XP002245583 ISSN: 0020-7136 abstract -----	1-16

## INTERNATIONAL SEARCH REPORT

International Application No

PCT/EP 03/00078

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
WO 0218448	A	07-03-2002	IT PD20000208 A1 AU 9181501 A WO 0218448 A2	28-02-2002 13-03-2002 07-03-2002
WO 0241877	A	30-05-2002	AU 3969702 A WO 0241877 A1 US 2003096734 A1	03-06-2002 30-05-2002 22-05-2003
WO 0218450	A	07-03-2002	IT PD20000207 A1 AU 1387002 A WO 0218450 A1 EP 1313772 A1	28-02-2002 13-03-2002 07-03-2002 28-05-2003
EP 0341745	A	15-11-1989	IT 1219587 B AT 115590 T AT 195534 T AU 631125 B2 AU 3574789 A CA 1339122 C DE 68919900 D1 DE 68919900 T2 DE 68929241 D1 DE 68929241 T2 DK 10990 A WO 8910941 A1 EP 0341745 A1 EP 0614914 A2 ES 2064378 T3 ES 2151910 T3 FI 107050 B1 GR 3015035 T3 GR 3034651 T3 HU 53666 A2 IL 90274 A JP 10324701 A JP 2504163 T JP 2941324 B2 NZ 229100 A US 5676964 A	18-05-1990 15-12-1994 15-09-2000 19-11-1992 29-11-1989 29-07-1997 26-01-1995 11-05-1995 21-09-2000 05-04-2001 12-03-1990 16-11-1989 15-11-1989 14-09-1994 01-02-1995 16-01-2001 31-05-2001 31-05-1995 31-01-2001 28-11-1990 12-09-1996 08-12-1998 29-11-1990 25-08-1999 28-08-1995 14-10-1997
WO 9961080	A	02-12-1999	IT PD980131 A1 IT 1302535 B1 AT 227589 T AU 748303 B2 AU 4368099 A CA 2332532 A1 DE 69903945 D1 DK 1085917 T3 WO 9961080 A1 EP 1085917 A1 ES 2184460 T3 JP 2002516154 T	29-11-1999 05-09-2000 15-11-2002 30-05-2002 13-12-1999 02-12-1999 19-12-2002 10-03-2003 02-12-1999 28-03-2001 01-04-2003 04-06-2002
US 5520916	A	28-05-1996	IT 1254704 B AT 200630 T AU 669147 B2 AU 3346693 A BG 98863 A	09-10-1995 15-05-2001 30-05-1996 19-07-1993 31-05-1995

## INTERNATIONAL SEARCH REPORT

International Application No

PCT/EP 03/00078

Patent document cited in search report		Publication date	Patent family member(s)	Publication date		
US 5520916	A		BG 302 Y1	31-05-1999		
			CA 2126085 A1	24-06-1993		
			DE 69231796 D1	23-05-2001		
			DE 69231796 T2	15-11-2001		
			DK 618817 T3	30-07-2001		
			WO 9311803 A1	24-06-1993		
			EP 0618817 A1	12-10-1994		
			ES 2155832 T3	01-06-2001		
			FI 942894 A	18-08-1994		
			GR 3036197 T3	31-10-2001		
			HU 68680 A2	28-07-1995		
			JP 7502430 T	16-03-1995		
			NO 942330 A	17-08-1994		
			NZ 246575 A	24-04-1997		
			PT 618817 T	30-08-2001		
			RO 115017 B1	29-10-1999		
			RU 2133127 C1	20-07-1999		
			US 5824335 A	20-10-1998		
		<hr/>				
		WO 0001733	A	13-01-2000	IT PD980169 A1	07-01-2000
AU 4639799 A	24-01-2000					
CA 2339066 A1	13-01-2000					
EP 1095064 A1	02-05-2001					
WO 0001733 A1	13-01-2000					
JP 2002519481 T	02-07-2002					
<hr/>						
WO 0054762	A	21-09-2000	AU 3886600 A	04-10-2000		
			CA 2365767 A1	21-09-2000		
			EP 1162984 A2	19-12-2001		
			JP 2002539157 T	19-11-2002		
			WO 0054762 A2	21-09-2000		
			US 2002169144 A1	14-11-2002		
			US 6472379 B1	29-10-2002		
<hr/>						
WO 0057896	A	05-10-2000	AU 4025500 A	16-10-2000		
			CA 2366604 A1	05-10-2000		
			EP 1165112 A1	02-01-2002		
			JP 2002540161 T	26-11-2002		
			WO 0057896 A1	05-10-2000		
			US 2003054991 A1	20-03-2003		
<hr/>						
US 4851521	A	25-07-1989	IT 1203815 B	23-02-1989		
			IT 1214658 B	18-01-1990		
			AT 135713 T	15-04-1996		
			AT 227741 T	15-11-2002		
			AU 591501 B2	07-12-1989		
			AU 5983686 A	26-02-1987		
			CA 1341276 C	31-07-2001		
			DE 3650501 D1	25-04-1996		
			DE 3650501 T2	21-11-1996		
			DE 3650776 D1	19-12-2002		
			DK 323686 A	09-01-1987		
			EP 0216453 A2	01-04-1987		
			EP 0696598 A1	14-02-1996		
			ES 2001512 A6	01-06-1988		
			FI 862878 A ,B,	09-01-1987		
			FI 892710 A ,B,	02-06-1989		
			FI 892711 A	02-06-1989		

## INTERNATIONAL SEARCH REPORT

International Application No

PCT/EP 03/00078

Patent document cited in search report	Publication date	Patent family member(s)	Publication date			
US 4851521	A	FI 94767 B	14-07-1995			
		HU 42512 A2	28-07-1987			
		IE 81120 B1	22-03-2000			
		IL 79362 A	31-07-1995			
		IN 165582 A1	25-11-1989			
		JP 2569012 B2	08-01-1997			
		JP 62064802 A	23-03-1987			
		KR 8701901 B1	21-10-1987			
		NO 862734 A , B,	09-01-1987			
		NO 910295 A	09-01-1987			
		NZ 216786 A	26-05-1992			
		NZ 233045 A	26-05-1992			
		PH 25189 A	27-03-1991			
		PT 82941 A , B	01-08-1986			
		SG 90006 A1	23-07-2002			
		US 4965353 A	23-10-1990			
		US 5202431 A	13-04-1993			
		US 5336767 A	09-08-1994			
		PH 25383 A	03-06-1991			
		PH 25382 A	03-06-1991			
		PH 25642 A	21-08-1991			
		PH 25643 A	21-08-1991			
		ZA 8605071 A	30-09-1987			
		EP 0466300	A	15-01-1992	US 5143724 A	01-09-1992
					AT 165979 T	15-05-1998
					AU 629467 B2	01-10-1992
					AU 7405591 A	09-01-1992
	CA 2041074 A1			10-01-1992		
	DE 69129391 D1			18-06-1998		
	DE 69129391 T2			08-10-1998		
	DK 466300 T3			07-10-1998		
	EP 0466300 A2			15-01-1992		
	ES 2117634 T3			16-08-1998		
	HK 1011502 A1			09-07-1999		
	JP 4261664 A			17-09-1992		
	JP 7093943 B			11-10-1995		
	US 5399351 A			21-03-1995		
	US 5246698 A			21-09-1993		

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